IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Examiner: Ganapathy KRISHNAN
De Ambrosi et al.	Group Art Unit: 1623
Application No.: 10/555,897	Confirmation No.: 4476
Filed: April 18, 2006	
For: Process for the Physical Depolymerization of Glycosaminoglycanes and Products Obtained Therefrom	Date: September 21, 2008

DECLARATION OF LUIGI DE AMBROSI UNDER 37 CFR §1.132

I, Luigi De Ambrosi, a citizen of Italy, residing at Carducci Street n°8-Santhià-(VC) Italy, hereby declare and state that:

1. I was educated (1940) at the Faculty of Medicine (University of Turin) for four years:

(Advisor for plasma proteins separation in Molinette Hospital – Prof. Bastai)

(Lecturer of Microbiology in Genaral Phatology Institute – Prof. Di Macco).

For war service I passed to the Faculty of Sience (Turin) from which I graduated with a degree in Pharmaceutical and Toxicological Chemistry (1948).

My pharmacological, scientific and thecnical background was performed at the following Pharmaceutical Firms :

SCHIAPPARELLI - Turin-Research Laboratory on Digital compounds (Prof. Migliardi)

MARXER INSTITUTE - Ivrea - Tubercolosis treatment with Isonicotinil Hydrazide (Prof. Marxer)

Customer No.: 27160

LA FARMOCHIMICA ITALIANA - Milan- Biological Manager

ISTITUTO SANT'ALESSANDRO - Santhià - Technical Manager for Opotherapic preparations

LABORATORI DERIVATI ORGANICI LDO - Trino - Technical Manager for extraction, separation, purification of: Heparin, Heparan, Dermatan, Chondroitin, Ferritin, Suprarenal, Liver and Plasma extracts. The first fisical depolimerisation with gamma rays of a LMM Heparin was patented in 1986 and a VLMM Heparin was patented in 2003 in collaboration with Loyola University of Chicago (Prof. Jawed Fareed)

During all these years I was in scientific relations with Pharmacological Institute of Siena (Prof. Giorgio Segre) and of Catania (Prof. Umberto Scapagnini) and with Ronzoni Institute of Milan (Prof. Casu – Prof. Torri)

I am currently employed as Honoraris President by LDO, where I am involved in Research and Development Section.

2. I have reviewed the subject application, including the pending claims, the Final Office Action mailed on April 21, 2008, as well as Balazs et al., "Irradiation of Mucopolysaccharides with Ultraviolet Light and Electrons," Radiation Research 11, 149-164 (1959) (hereinafter "Balazs") cited against the claims of the above-referenced patent application.

3. I am a named inventor on the subject application, which claims a dynamic irradiation process for the depolymerization of heparin wherein the depolymerized heparin has a M_w less than or equal to 50% of the original M_w of said heparin prior to depolymerization, said dynamic irradiation process comprising exposing said heparin in solution at a concentration between 2 and 25% w/v to UV radiation having a peak of from 245 nm to 260 nm for a sufficient time to

reduce the M_w of the depolymerized heparin by at least 50% as compared with the M_w of said heparin prior to said exposure to UV radiation. *See* Claim 1.

4. In contrast to the process of Claim 1, Balazs fails to teach or suggest a dynamic irradiation process in which a solution to be irradiated is circulating as a thin layer in a lamp jacket and then returns to a reservoir. Instead, Balazs discloses static irradiation in which samples were irradiated in either quartz cells or Teflon containers having silica windows. *See* Balazs at p. 150.

5. Claim 1 of the subject application recites that the heparin is in solution at a concentration between 2 and 25% w/v. In contrast, the Balazs process operates at much lower concentrations, i.e., concentrations of about 0.1%. *See* Balazs Fig. 2. Balazs fails to teach or suggest the claimed heparin concentration of Claim 1.

6. For reasons that are not entirely clear, the dynamic irradiation processes of the present invention form compositions having different molecular weight distributions from those formed by the Balazs process. The two processes form different products.

7. Balazs reports cationic dyebinding values of dialyzed and undialyzed heparin at time 0, after 120 minutes and after 360 minutes of irradiation. *See* Balazs p. 153, Table 1.

8. Cationic dyebinding is a method to determine the amount of anions present in a molecule.

9. One characteristic of cationic dyebinding is that the method does not take into account small molecules (e.g., on the order of less than 1,000 Da) that do not precipitate after reaction with the cation.

10. Table 1 of Balazs provides lower values for dialyzed heparin than undialized heparain at 120 and 360 minutes after irradiation. These results suggest that the membrane used for dialysis in Balazs removed fragments of heparin that were still capable of precipitating by cationic dyebinding. Those skilled in the art would appreciate that the removed fragments would have a Mw less than 1000 Daltons.

11. In addition, Table 1 indicates that cationic dyebinding values decreased significantly as irradiation time increased for undialyzed heparin. Specifically, the cationic dyebinding value for undialyzed heparin decreased from 3.90 to 1.59—a reduction of nearly 60%. This pattern suggests that a large amount of very small fragments (less than 1000 Daltons) were formed during the Balazs irradiation process, which fragments did not precipitate during cationic dyebinding.

12. The characteristics recited in Table 1 of the Balazs suggest that the Balazs process forms a product having a large fraction of fragments having a Mw less than 1000 Da.

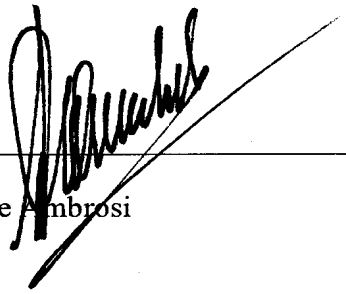
13. The processes of the present invention form fragments having a Mw less than 1000 Da in an amount less than 6%, even when the overall Mw has been reduced to less than 5000 Da.

14. One skilled in the art would expect that cationic dyebinding values of irradiated heparin according to the processes of the present invention would be much higher than those reported in Balazs. This is a surprising and unexpected result of the process of the present invention.

15. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and

further, that these statements were made with the knowledge that willful false statements and the

like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Luigi De Ambrosi

25 September 2008

Date

Customer No.: 27160